# MORPHOLOGY AND PATHOMORPHOLOGY

# Effect of Hydra Morphogen Peptide on the Dynamics of Tissue Components of the Myocardial Layers in Compensatory Hypertrophy of the Heart

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Morphological differences were noted previously in myocardial layers of rat ventricles [2], and their nonuniform reaction to a heavy load due to a coarctation was demonstrated 10 days postoperation [3].

The aim of the present investigation was a morphological study of the effect of a single injection of synthetic hydra morphogen peptide (HMP) on the dynamics of the tissue components of different myocardial layers in the development of myocardial hypertrophy in rats.

## MATERIALS AND METHODS

The experiments were carried out on 12 male Wistar rats weighing 180-200 g. HMP was dissolved in saline and administered i.p. in a dose of 20 μg/kg (1 ml) once immediately after the operation. After the operation the control animals received an injection of saline with an equimolar mixture of amino acids. A 50% coarctation of the abdominal aorta was performed as described previously [3]. Three, 10, and 24 days after treatment the thorax was opened under nembutal anesthesia and the heart was perfused with 2% glutaraldehyde in 0.1 M phosphate buffer and then removed. The myocardial plates, excised from the middle third of the left ventricle, were fixed, dehydrated, and embedded in Epon. The semithin sections, stained with toluidine blue, were measured under the microscope at a final magnification of 1350, using a multipurpose ocular morphometric grid [1]. Morphometric analysis revealed no difference between the series of treated animals which did and did not receive the amino acid mixture, and therefore the control data are not listed in the Table.

The following parameters were determined: the relative volume of myocytes $V_m$; relative volume of connective tissue $V_{ct}$; relative volume of capillaries $V_c$; surface area of myocytes $S_m$; surface area of capillaries $S_c$. In addition, the average diameter of the cardiomyocytes $D_m$ was also calculated. An Amstrad PC 1640 computer was used for the calculations and statistical analysis by the *t* test, allowing for the primary and secondary objects.

## RESULTS

The results of the measurements are listed in Table 1. The findings attested that the new level

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TABLE 1. Dynamics of Tissue Component Indexes in Different Left Vetricle Myocardial Layers of Rat Heart during Formation of Hypertrophy and during Hypertrophy under HMP Action

| Parameter | Norm | | Subendocardial layer after | | |
|---|---|---|---|---|---|
| | | | 3 days | 10 days | 24 days |
| $V_m$ | 0.820±0.01 | a. a. | 0.750±0.00 | 0.710±0.01 | 0.760±0.01 |
| | | HMP | 0.790±0.01** | 0.800±0.01** | 0.803±0.01 |
| $S_m$ | 48.56±0.85 | a. a. | 42.31±0.43 | 38.60±2.90** | 38.81±1.21 |
| | | HMP | 39.23±0.62 | 31.95±1.66** | 34.63±0.37 |
| $V_c$ | 0.063±0.00 | a. a. | 0.070±0.01 | 0.077±0.01 | 0.080±0.00 |
| | | HMP | 0.064±0.00** | 0.067±0.00 | 0.072±0.00 |
| $S_c$ | 9.84±0.28 | a. a. | 13.06±0.35 | 18.05±1.38 | 14.87±1.72 |
| | | HMP | 10.30±0.45** | 11.75±0.63** | 12.30±0.32 |
| $V_{ct}$ | 0.173±0.01 | a. a. | 0.220±0.01 | 0.283±0.01 | 0.301±0.00 |
| | | HMP | 0.190±0.00 | 0.203±0.00 | 0.280±0.01 |
| $D_m$ | 12.70±0.02 | a. a. | 12.30±0.32** | 12.04±0.43 | 12.90±0.14 |
| | | HMP | 12.90±0.16** | 13.50±0.53** | 13.60±0.17** |
| $S_m/V_m$ | 54.90±0.67 | a. a. | 56.41±0.76 | 47.07±0.35 | 51.07±0.27 |
| | | HMP | 49.66±0.49 | 54.40±0.43 | 43.29±0.67 |
| $S_m/S_c$ | 4.93±0.05 | a. a. | 3.24±0.08 | 2.14±0.02 | 2.60±0.07* |
| | | HMP | 3.81±0.12 | 2.14±0.05 | 2.81±0.04 |
| $V_m/V_c$ | 12.95±0.50 | a. a. | 10.71±0.43 | 10.65±0.36** | 9.50±0.28 |
| | | HMP | 12.34±0.35 | 9.22±0.44 | 11.11±0.18 |
| $V_c/V_{ct}$ | 0.41±0.03 | a. a. | 0.32±0.07 | 0.27±0.02* | 0.27±0.04*,** |
| | | HMP | 0.34±0.11 | 0.27±0.03 | 0.26±0.06** |
| | | Intramural layer | | | |
| $V_m$ | 0.720±0.01 | a. a. | 0.730±0.01** | 0.760±0.01* | 0.764±0.01** |
| | | HMP | 0.750±0.01 | 0.800±0.01 | 0.810±0.00 |
| $S_m$ | 48.68±0.87 | a. a. | 44.70±2.36* | 40.15±3.24* | 39.50±0.78* |
| | | HMP | 42.38±1.23 | 38.35±2.38 | 37.26±1.13** |
| $V_c$ | 0.132±0.01 | a. a. | 0.108±0.00 | 0.073±0.01 | 0.096±0.01*,** |
| | | HMP | 0.103±0.00 | 0.060±0.01 | 0.098±0.01** |
| $S_c$ | 19.70±0.31 | a. a. | 15.80±0.34* | 10.60±0.90* | 16.63±0.14* |
| | | HMP | 15.40±0.28 | 10.60±0.83 | 17.10±0.72 |
| $V_{ct}$ | 0.273±0.01 | a. a. | 0.253±0.00 | 0.237±0.01 | 0.250±0.01* |
| | | HMP | 0.190±0.01 | 0.205±0.01** | 0.280±0.01 |
| $D_m$ | 11.88±0.02 | a. a. | 12.70±0.23* | 13.80±0.41* | 13.92±0.36* |
| | | HMP | 12.72±0.31 | 13.81±0.26 | 13.87±0.25** |
| $S_m/V_m$ | 62.60±0.38 | a. a. | 61.23±0.43 | 52.83±0.19 | 51.70±0.24 |
| | | HMP | 56.51±0.62 | 47.81±0.75 | 46.00±0.58 |
| $S_m/S_c$ | 2.48±0.06 | a. a. | 2.83±0.06* | 3.79±0.09* | 2.38±0.04* |
| | | HMP | 2.75±0.07 | 3.61±0.11 | 2.18±0.14 |
| $V_m/V_c$ | 5.76±0.37 | a. a. | 6.72±0.32 | 10.41±0.28 | 7.92±0.18 |
| | | HMP | 7.28±0.21 | 13.33±0.14 | 8.26±0.38 |
| $V_c/V_{ct}$ | 0.51±0.05 | a. a. | 0.43±0.07* | 0.31±0.03*,** | 0.38±0.04* |
| | | HMP | 0.54±0.04 | 0.29±0.02 | 0.35±0.08 |
| | | Subepicardial layer | | | |
| $V_m$ | 0.699±0.01 | a. a. | 0.740±0.01 | 0.790±0.01* | 0.810±0.01 |
| | | HMP | 0.710±0.00 | 0.690±0.01 | 0.780±0.01 |
| $S_m$ | 58.62±1.18 | a. a. | 50.82±1.45 | 35.20±2.40 | 37.47±1.90 |
| | | HMP | 53.35±0.95 | 39.80±2.50** | 41.36±1.63 |
| $V_c$ | 0.169±0.01 | a. a. | 0.152±0.00 | 0.143±0.01** | 0.130±0.01* |
| | | HMP | 0.130±0.00 | 0.110±0.01** | 0.122±0.00 |
| Sc | 27.30±0.50 | a. a. | 24.23±1.21* | 18.00±1.42 | 21.14±1.45* |
| | | HMP | 23.27±0.81 | 14.90±0.75 | 24.52±1.18 |
| $V_{ct}$ | 0.302±0.01 | a. a. | 0.275±0.01 | 0.212±0.01* | 0.260±0.01 |
| | | HMP | 0.303±0.01** | 0.310±0.01** | 0.290±0.01 |
| $D_m$ | 9.28±0.02 | a. a. | 9.57±0.03* | 10.30±0.40*,** | 10.50±0.08 |
| | | HMP | 9.75±0.11 | 10.96±0.41** | 11.42±0.06 |
| $S_m/V_m$ | 79.80±0.29 | a. a. | 68.68±0.21 | 44.56±0.55 | 46.26±0.19 |
| | | HMP | 75.14±0.39 | 57.73±0.29 | 53.02±0.43 |
| $S_m/S_c$ | 2.15±0.10 | a. a. | 2.10±0.05** | 2.00±10.07 | 1.77±0.08* |
| | | HMP | 2.29±0.04 | 2.67±0.14 | 1.69±0.03 |
| $V_m/V_c$ | 4.13±0.25 | a. a. | 4.87±0.23* | 5.53±0.17 | 6.23±0.14* |
| | | HMP | 5.46±0.31** | 6.27±0.65** | 6.39±0.43 |
| $V_c/V_{ct}$ | 0.57±0.03 | a. a. | 0.55±0.04 | 0.68±0.02 | 0.50±0.06* |
| | | HMP | 0.43±0.05 | 0.35±0.03 | 0.42±0.03 |

Note. The differences of amino acid control (a.a.) from HMP administration: * $p>0.05$, in other cases $p<0.05$; differences between adjacent periods: ** $p>0.05$, in other cases $p<0.05$.

of cardiac functioning during the development of compensatory hypertrophy of the myocardium is characterized by pronounced zonal changes in the quantitative indexes of tissue components. A de-

creased contribution of myocyte volume was noted in the subendocardial layer, but a tendency toward normalization, appeared at later periods of the experiment. Administration of HMP resulted in the stabilization of this parameter at a normal level as soon as the 10th day postoperation. Later the peptide did not manifest an effect. The total area of the cardiomyocytes was HMP-dependent and was lower throughout the experiment than in the control, but the dynamics of this parameter was preserved. The diminishment of the cardiomyocyte area during the development of myocardial hypertrophy is related to an increase of cell size [7], this being consistent with the data on the dynamics of the average diameters. The tendency for cardiomyocyte surface to increase at later periods has been attributed to the capacity of muscle cells to ramify [5]. Pronounced changes were noted in the capillaries of the subendocardial myocardium, namely, both $V_c$ and $S_c$ rose. Injection of HMP reduced the manifestation of these alterations at all experimental periods, which may be due to more adequate cardyomyocyte functioning under the influence of the peptide. The reduced edema of the interstice and subsequent changes of the connective tissue share in the early development of hypertrophy probably stem from the reduced blood supply of the subendocardial layer in peptide-affected myocardium. The increase of the contribution of the connective tissue component at later periods is reportedly explained by its advanced growth [4]. In the intramural layer in the course of development of heart compensatory hypertrophy, the changes noted were sometimes opposite to the ones described in the subendocardial layer. The fraction of cardiomyocytes in this layer steadily rose in all periods under study. The peptide effect was weakly expressed after 3 days; after 10 days it resulted in a reliable increase of the muscle cell share, which did not alter subsequently. One of the causes of such results may lie in the augmentation of myocyte size in this myocardial layer, as is also attested by the increase of average cell diameter and the decrease of the relative area. The effect of HMP on the changes of these indexes is weakly manifested (the differences are not significant). The alterations noted in the capillary system may also be responsible for the increase of the myocyte share. In the intramural layer at all experimental periods there was a reduction of their volume and area. Data on a decrease of these indexes have been reported, but without an indication of specific myocardial layers [4]. This may testify to a redistribution of the circulation between the layers during the process of formation of myocardial hypertrophy. Evidence of the possibility of such a redistribution has also been obtained using physiological techniques [5,6]. An increase of the cardiomyocyte share followed by a diminishment of their total area was noted in the subepicardial layer during the development of compensatory hypertrophy. Peptide administration retards the pace of growth of the myocyte fraction during the period up to 10 days (the differences from the norm are not significant), but this index rises later. Under HMP action $S_m$ decreases to a lesser degree and stabilizes earlier. An increase of $D_m$ was obtained only on the 24th day, possibly due to a lesser involvement of this layer to the myocardial load in the early stages of the development of heart hypertrophy. The basis of such a phenomenon may be an improved functioning of the HMP-affected intramural layer. Regular changes were noted in regard to the capillary system, i. e. $V_c$ and $S_c$ declined. After 10 days the $V_c$ changes stabilized while the $S_c$ variation reached its maximum, there was a slight rise of this index later. The effect of HMP manifested itself in a more reliable decrease of $V_c$ in the early stages and in its stabilization at a lower level. A significant decrease of $S_c$ under peptide administration appeared only toward the 10th day. The changes found in the capillary system attested to the fact that the subendocardial myocardium was also drawn into the reaction of circulatory redistribution between layers. There was some decrease of the volume share of connective tissue in the subepicardial layer of the myocardium, which evidently related to the augmentation of $V_m$. Injection of HMP somewhat stabilized this index in all periods studied.

Thus, a single injection of HMP facilitates the development of myocardial hypertrophy at early periods, reducing the gravity of morphological changes and, probably, affecting the times of their development.

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